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Impact of filter paper on fluorescence measurements of buffered saline filtrates

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Abstract

Fluorescent contaminants have been observed when stock solutions of phosphate buffered saline solutions at each of three pH values (2.2, 7.5, and 12.5) are analyzed after passing through commercially available filter paper. The filtrate's fluorescence was observed to exhibit a maximum signal at 440.0 nm when excited at 365 nm. Detection of trace components could have significant implications in the design and implementation of sample processing protocols when using fluorescence.

1. Introduction

During the development of sample processing protocols for investigating plant extracts using molecular fluorescence spectroscopy, common filter paper was used to physically separate ground plant material from the resulting solution. These materials were suspend in a phosphate buffered saline (PBS) solutions adjusted to each of three pH values (2.2, 7.5, and 12.5).

Excitation of the filtrate at 365 nm yielded an emission spectrum from 375 to 620 nm that has been implicated in the identification of plant species in pre- and post-digestion samples [1]. It was initially assumed that these aqueous filtrate solutions would not be impacted by exposure to a cellulose-based paper filter. Unfortunately, closer spectroscopic observation of the resulting filtrates demonstrated this assumption to be overly optimistic. This note is intended to inform other researchers using this common filter paper material of this potential source of background emission.

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2. Experimental

Briefly, the experimental configuration used a 500 W Xe/Hg-arc lamp with a 1/4 m monochromator (Instruments SA, model HR-20) for excitation at 365 nm. Sample solutions were each transferred to a 1.0 cm quartz cuvette for analysis. The emitted radiation was focused onto the entrance slit of a 1.0 m focal length monochromator similar to that described elsewhere [2] with photomultiplier tube detection (Hamamatsu, R955). Signal intensities were subsequently digitized and recorded by a microcomputer system using software developed in house with LABVIEW™ (National Instruments, Austin, TX).

Samples from three lots of Whatman™ No. 4 filter paper were used in this study (lots 1005, 01157, and 813132). Samples were prepared by passing three separate 10 ml aliquots of the PBS solution through the un-wetted filter paper. Fig. 1 shows the mean fluorescence spectra recorded as a function of solution pH for filter paper samples from each of the three lots investigated (b–d, respectively). The significant spectral feature at 418.0 nm within the spectra shown in Fig. 1 has been assigned to a characteristic water Raman band at 3450 cm^{-1} [3].

3. Results and discussion

Each of two solutions were generated and probed for their contribution to the measured fluorescence signals: the pH adjusted PBS solutions [1] before and [2] after each solution was passed through the filter paper. In an effort to determine the reproducibility of this phenomenon, samples were prepared in triplicate for each lot of paper used at each PBS solution pH. Additionally, each piece of filter paper was exposed to three successive aliquots of the PBS solution and each of these filtrates were collected and subsequently analyzed. The fluorescence signal from each stock PBS solution used in the preparation of these samples was recorded. In an effort to identify the source of the observed fluorescence signals resulting from these solutions, all materials involved in sample processing (i.e. the Latex gloves used by the investigator, the PBS solutions, and the glass funnel and test tubes) were investigated. Because only those solutions that had passed through the filter paper exhibited a measurable fluorescence signal, it was concluded that the filter paper used was the only source of this background signal.

These other possible sources of fluorescent contamination (e.g. gloves, glassware, cuvettes, and unfiltered solutions) were each systematically investigated and subsequently eliminated from further consideration due to the absence of any detectable fluorescence signals. It should be emphasized that samples from only three lots of the Whatman™ No. 4 filter paper were tested. Although the filter paper contained in the boxes evaluated had not been contaminated prior to this study, they do not constitute a valid statistical sampling of these lots. Similarly, the results of these studies cannot be extrapolated to other lots or other types of filter paper including those from this manufacturer.

These data reveal the extraction of fluorophores at low, neutral and high pH from the filter paper samples. These materials were found in filtrates from each of the three paper lots studied. To determine a possible procedure for the removal of these potentially interfering fluorescent species, an additional study was conducted to evaluate how subsequent washings through a single filter paper would affect the observed 'contaminant' fluorescence signal intensity. Fluorescence spectra from filtrate solutions resulting from each of three 10 ml aliquots of the PBS solutions were recorded in triplicate for the different single filter papers. The first 10 ml portion was contacted with the unwetted filter paper while the second and third aliquots were passed through the already wetted paper.

The presence of the fluorophores in the filtrates varied with paper lot number. The intensity of the fluorescence band at 440 nm was observed to be dependent on solution pH with the greatest signal magnitude recorded with the pH 7.5 PBS solution. This might be explained by the deprotonation of the responsible fluorescent molecules in the pH 7.5 solution relative to the acid solution condition (pH 2.2). This could then either alter the extraction efficiencies of the responsible compound(s) or

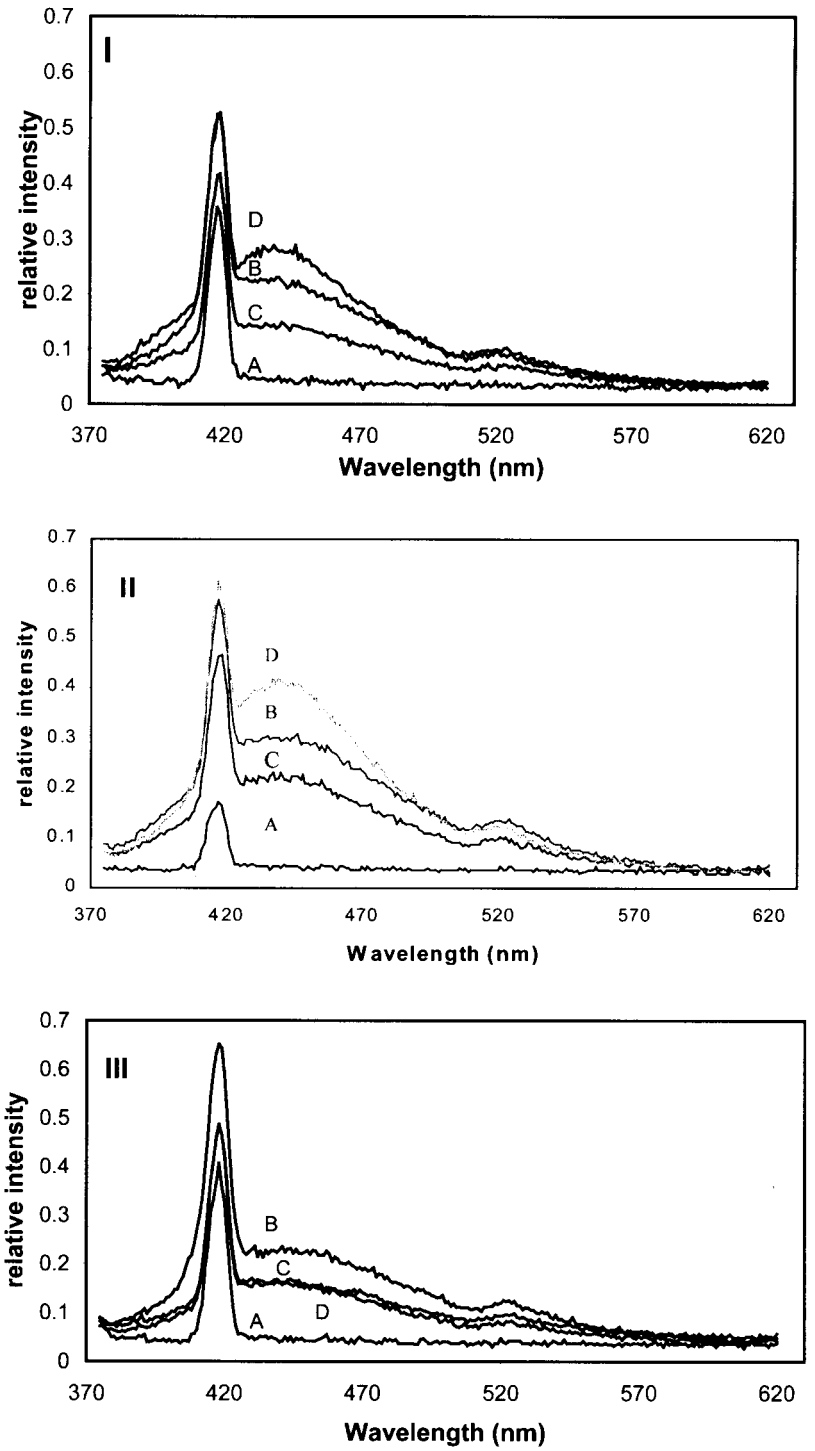

Fig. 1. Measured fluorescence intensities as a function of emission wavelength for the (A) PBS solution, unfiltered; (B) buffer solution passed through filter paper lot 1005; (C) passed through paper lot 01157; (D) passed through paper lot 813132 with solution pH values of (I) 2.2, (II) 7.5, and (III) 12.5.

result in an increase in absorbance at 365 nm. The interpretation of this data is further complicated by an observed decrease in the relative emission intensity of this material when the pH was further increased to 12.5. Fortunately, sequential exposure of each filter paper sample to a few as three aliquots of each solution resulted in sufficient decrease in the measured fluorescence signal for the purposes of the current investigations but was not able to completely remove the fluorescing species.

The reactivity of these extractable fluorescent contaminates was not determined and are beyond the scope of this technical note. These data are presented to bring awareness of this potential complication when using filter paper in sample preparation procedures for fluorescence determinations. The impact of these materials can be minimized, or eliminated through several washings of the filter paper prior to its use. Again, these results are based on only three different lots of filter paper. The presence of such fluorescent species on other types or lots of paper must be determined experimentally.

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